

THE PROLONGED GESTATION PERIOD IN SUCKLING MICE

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INTRODUCTION

A short paper by Prof. J. F. Daniel ('10) on white mice, and a later paper by Dr. H. D. King ('13) on white rats, have shown that in these two animals the gestation period is lengthened when the females suckle young during pregnancy. Daniel found a fairly definite relation existing in mice between the degree of prolongation of the gestation period and the number of young being suckled, about one day being added to the period of pregnancy for each of the nursing young. King, on the contrary, found that in rats the gestation period is normal "if the female is suckling five or less young and is carrying five or less young," while "the gestation period may be prolonged from one to six days if an albino female, suckling five or less young, is carrying six or more young," and "the period of gestation is always prolonged when a female is suckling six or more young."

Professor Daniel offered no explanation of the prolongation of the gestation period in suckling white mice other than stating the possibility that it might be due to lactation, either delaying ovulation or generally slowing down embryonic development, with the latter as the more probable. Dr. King, however, states positively that ovulation in albino rats is not delayed by lactation, but that the suckling of a litter that contains six or more young seems to lessen the food supply to the foetal young and so retards their development.

The present work has been in progress for nearly five years, and certain phases of it are still being investigated. White

mice only have been used, and the problem of prolonged gestation in suckling females has been approached from the embryological standpoint in the attempt to determine the detailed facts involved.

It was evident at the start that exact criteria for determining the stage of development of any embryo must be established, and, while Sobotta ('95) gives the time relations of development from ovulation to implantation, the series of eggs and embryos of known age prepared especially for this work includes sets from both suckling and non-suckling females, spaced at about twenty-four hour intervals from the time of parturition to the birth of the following litter. In each case the age of the specimens was definitely established by placing in individual cages all pregnant females, together with a male, looking every day for new litters, removing the male in every instance the day following the birth of the litter, and, if the female was to be used for the non-suckling series, removing all the young as soon as found. The age of eggs and embryos found in these females was then reckoned from the birth of the preceding litter, e.g., the day the litter was found is the first day after parturition.

THE DEVELOPMENTAL CYCLE IN NON-SUCKLING FEMALES

The development, in non-suckling white mice, of eggs and embryos is usually as indicated below, the exception being the very rare instances where the males probably paired with the females much earlier than the average time, following parturition, and in consequence the developing eggs or embryos are slightly ahead of this schedule. Cases of this kind number only three in eighty sets of eggs and embryos studied. It should be noted, further, that as a rule (one exception in fifty instances) when the males were left with the females the state of development of eggs and embryos was the same as in cases where the males were removed twenty-four hours after parturition. The single exception found was a set of normal eggs in the two-cell stage found in a female six days after parturition, a male having been in the cage with her continuously since a time preceding the birth of the litter.

Parturition. White mice give birth to litters at all hours of the day and night, but much more commonly at night or, according to Long and Mark ('11), in the early morning.

1 day post-partum. If the female white mouse comes in heat, as is the rule during the warmer part of the year (April to October) and the new born young are not suckled, within twenty-four hours after parturition ovulation and pairing (provided a male is present) will occur.

2 days post-partum. The eggs have been fertilized in the upper third of the Fallopian tubes and the first cleavage has occurred.

3 days post-partum. The eggs are still in the two-cell stage.

4 days post-partum. Cleavage is again in progress and morulas of 8 to 10 blastomeres are found at this time.

5 days post-partum. Morulas of 12 to 16 blastomeres. At the close of this day the morulas develop a central cavity, thus becoming blastulas, and at the same time they pass from the Fallopian tubes into the horns of the uterus.

6 days post-partum. Blastodermic vesicles lie free in the horns of the uterus.

7 days post-partum. The blastodermic vesicles are now implanted in proliferated masses of uterine cells which completely obstruct the lumen. The embryos themselves are in the 'egg-cylinder' stage, the 'cylinder' almost filling the vesicle and possessing a single, undivided cavity.

8 days post-partum. The egg-cylinder in embryos of this age has its lumen divided into three cavities.

9 days post-partum. The embryo now possesses a medullary groove, which is open except at the extreme anterior end.

10 days post-partum. Embryos of this age have the medullary groove closed in its anterior half, and for the first time show a heart.

11 days post-partum. The medullary groove is now closed for more than half its length; the optic vesicles are budding off from the brain; and the auditory vesicles appear as cup-shaped depressions in the ectoderm.

12 days post-partum. The medullary groove has closed except at the extreme posterior end; the auditory vesicles are almost or

entirely closed. The fore limb buds are present, together with the first nephric tubules and the anlage of the liver.

13 days post-partum. At this age the embryo is a decidedly complex organism, and from this time on the daily changes are rather matters of detail than the appearance of entirely new structures. The characteristic features of this particular stage of development are these: a well developed cranial flexure; optic vesicles completely separated from the brain, invaginated, and showing the beginnings of lens formation. The nasal capsules are visible; the liver has developed into a distinct organ; hind limb buds are present. The embryo has the anlagen of the lungs, and a few pancreatic tubules; the auditory vesicles have withdrawn from the surface and are connected by nerve fibers with the brain. Along the free border of the kidneys, especially at the posterior end appears the genital ridge with a few large cells with large round nuclei, the primordial germ cells, scattered through a much larger number of smaller, epithelial cells.

14 days post-partum. The eyes have developed to the stage where the lenses have a solid, clear core. Dense masses of connective tissue foreshadow the future location of the bones of the limbs, girdles, and ribs. Whisker follicles are present; also the semi-circular canals of the ears. The kidneys possess definite boundaries. The nuclei of the red blood corpuscles are smaller and stain less deeply than in earlier stages, while their cytoplasm shows a faint indication of haemoglobin. The first indications of teeth follicles are found in embryos of this age; also the anlagen of the thymus and thyroid glands. The gonads differ from the preceding stage merely in having more of the primordial germ cells in the genital ridges.

15 days post-partum. Embryonic cartilage cells constitute the most striking characteristic of this stage, clearly differentiating embryos of this age from all younger specimens. Other features are the fewer and smaller blood spaces in the liver, as compared with fourteen-day embryos; the deeper straw yellow color in the cytoplasm of the red blood corpuscles, together with a few which are non-nucleated; the well-developed anlagen of

the teeth; and the presence of cartilage cells in the floor of the cranium. Sexual differentiation is present in embryos fifteen days post-partum, male specimens showing gonads in which follicle formation has already started, while the female gonads preserve the earlier condition of primordial germ cells scattered through a mass of epithelial cells.

16 days post-partum. Differential characteristics now become still more matters of detail and of direct comparison with earlier stages, however, embryos of this age differ from all younger ones in having a decidedly transparent cornea. The heart has assumed its final shape. The pancreas is a clearly defined organ. The nasal capsules open into the front part of the mouth, while the naso-pharynx is connected with both the nasal capsules and the back part of the mouth. The gonads show no marked change from those of fifteen days embryos.

17 days post-partum. The chief characteristic of embryos of this age is the commencement of ossification around the rib cartilages. Nucleated red blood corpuscles are very scarce. The tongue possesses conspicuous striated muscle cells, stratified epithelium, and at least one circumvallate papilla. The nasal capsules have lost their direct connection with the mouth, but the nasopharynx opens into both the anterior and posterior regions of the mouth. The anlagen of the cartilaginous rings of the trachea are present. The testes have tubules with a peripheral layer of small cells while the larger primordial germ cells occupy the lumen. In the ovaries an ingrowth of connective tissue is noticeable.

18 days post-partum. Ossification of the cartilaginous skeleton is now the striking feature, and the membrane bones of the upper jaw, hard palate and roof of the skull are also being formed. The testes show a considerable amount of connective tissue between the tubules, while the ovaries differ from those of the preceding stage only in that they project further into the abdominal cavity.

19 days post-partum. The ribs of embryos of this age have an outer shell of bone, and the underlying cartilage is being torn down to make a marrow cavity. There is a marked spongy

structure in the lungs; the eye balls show a differential curvature in the cornea and sclerotic; the naso-pharynx no longer opens into the front of the mouth. The testes show no change, but the ovaries are more spherical, and the ingrowth of connective tissue has forced most of the germ cells toward the periphery.

20 days post-partum. The iris and choroid of the eyes first show pigmentation at this time, and lymphoid tissue appears in the tonsils. One litter of four animals was born twenty days after the birth of a preceding litter and grew to maturity.

21 days post-partum. The feature of this stage of development is the ossification of the metacarpals and metatarsals. The testes have a well organized tunica albuginea and show less space between the individual tubules than in twenty-day specimens, while in the ovaries the primordial germ cells, or oogonia, are of varying sizes, the largest of them beginning to form follicles about themselves.

22 days post-partum. The embryos in non-suckling white mice have now completed their intra-uterine development, and parturition occurs.

Such, in brief, is the record of development from day to day of white mouse embryos carried by females not suckling young. More extended study and more material would certainly yield many more details, but the above data are sufficient basis for estimating the age of embryos of unknown history, provided only they come from non-suckling females. Also the evidence collected is adequate for comparison with the facts noted below concerning the development of embryos carried by suckling mothers.

THE DEVELOPMENTAL CYCLE IN SUCKLING FEMALES

White mice are able to become pregnant while lactating but when suckling a litter only about one female in five undergoes a complete pregnancy; those which do not complete a pregnancy either failing to ovulate (the majority of cases) or the fertilized eggs developing normally until shortly after implantation in the uterus, when they die and are absorbed (the minority of

cases). In some instances we find another state of affairs, certain ones of a set of implanted embryos undergoing normal development while others die and are absorbed, a very rare condition in non-suckling females.

Suckling females which are not going to skip an ovulation cycle shed their eggs within twenty-four hours of parturition as do non-suckling females. These eggs are then fertilized and divide according to the same time scheme as given above for eggs in non-suckling females, being in the two-cell stage on the second and third days following parturition, morulas on the fourth and fifth days, and blastulas lying free in the uterus on the sixth day post-partum.

Now comes the point of greatest interest in this investigation. The fertilized eggs in non-suckling females, as stated earlier in this paper, become implanted in the uterus at the close of the sixth day post-partum, the fertilized eggs in suckling white mice, on the contrary, lie free in the lumen of the uterus from the sixth to the end of the fourteenth day following parturition. The material on which this statement is based comprises serial sections of the entire uterus of ten females, suckling from three to eight young, killed at various times from the sixth to the fourteenth day post-partum, all of which show normal blastulas lying free in the uterus, with no sign of any reaction in the adjacent cells of the uterine epithelium (table 1).

TABLE 1

Data regarding all suckling white mice from which embryos were obtained from the sixth to the fourteenth day following parturition

AGE IN DAYS POST-PARTUM	STAGE OF DEVELOPMENT	NUMBER OF SUCKLING YOUNG
6	Free blastulas in uterus	6
7	Free blastulas in uterus	4
8	Free blastulas in uterus	5
9	Free blastulas in uterus	3
10	Free blastulas in uterus	5
10	Free blastulas in uterus	8
11	Free blastulas in uterus	6
12	Free blastulas in uterus	4
13	Free blastulas in uterus	4
14	Free blastulas in uterus	5

Here evidently is one, and perhaps the main cause of the prolonged gestation in suckling white mice. Ovulation, fertilization and early cleavage stages occur at the same time intervals in both suckling and non-suckling animals, but in the suckling females the uterine cells will not react to the eggs and enable the latter to become implanted while the mammary glands are withdrawing the surplus nourishment from the parent organism. In support of this statement it should be said that young mice derive all of their nourishment from the mother for the first ten or eleven days after they are born, and thereafter appear to nurse as much and as often as the female will let them, a fact which undoubtedly accounts for the precise number of suckling young exerting a definite influence on the rate of growth of intrauterine embryos.

If only one or two young mice are suckling the development of eggs and embryos proceeds as though none were suckling, but if three or more young nurse the gestation period is lengthened, according to both Daniel ('10) and to the small amount of evidence on this point possessed by the present writer, approximately one day for each animal suckled. This observed fact is, however, very difficult to correlate with the series of embryos obtained from females suckling three to eight young and killed from fifteen to twenty-four days after parturition (table 2). These embryos show a variation in state of development which appears to vary neither with the number of young suckled nor with the number of embryos carried; in fact it would seem as though in one instance (female killed eighteen days post partum table 2) that the embryos, being as fully developed as in a non-suckling female of the same age post-partum, would have come to birth on the twenty-second day following the previous parturition in spite of there being three young suckling. Even supposing this had happened, were all the facts known such an exception would probably be explainable, since occasional females may reasonably be expected to possess amounts of nourishment far in excess of the average, and some litters of young may start eating grain at an earlier age than usual. More difficult of explanation are such cases as the embryos of a female killed seventeen days

TABLE 2

Data regarding all suckling white mice from which embryos were obtained from the fifteenth to the twenty-fourth day following parturition. Embryos labeled (small) would probably all have been absorbed

AGE IN DAYS POST-PARTUM	DEVELOPMENT EQUAL TO EMBRYOS OF NON-SUCKLING ♀s OF	NUMBER OF SUCKLING YOUNG	NUMBER OF EMBRYOS
	<i>days p. p.</i>		
15	8	8	11
16	12	4	8
17	14	5	2 large + 6 small
18	18	3	9
19	12	7	2 large + 4 small
20	8	6	5
21	8	5	9
21	12	8	9
22	8	?	10
23	12	7	9
24	14	4	6 large + 1 small

post-partum (table 2) which, if, as we have every reason to believe, they became implanted at the close of the fourteenth day post-partum must in the course of the three days following have undergone a development which in embryos in non-suckling females requires eight days to complete, and this in spite of there being five suckling young.

At the present time work is in progress with a view to explaining, if possible, these apparent contradictions and until that work is completed, which may not be for some time, it does not seem desirable to attempt any further analysis of the facts presented in table 2.

SUMMARY

1. The present work has brought together sufficient data with which to determine, within the possible error of one day, the age of all embryos obtained from non-suckling white mice.

2. Ovulation, fertilization, and the early cleavage of the eggs bear the same time relations to parturition and to one another in both suckling and non-suckling white mice except the former are much more apt to skip an ovulation period.

3. Implantation of embryos in the uterus occurs in non-suckling white mice on the fifth day following parturition (provided the female did not skip an ovulation cycle).

4. Implantation of embryos in the uterus occurs in suckling white mice, with 3 or more young, on the fourteenth day following parturition (provided the female did not skip an ovulation cycle). In these lactating females the blastulas lie free in the lumen of the uterus from the sixth to the fourteenth day post-partum due supposedly to the activity of the mammary glands.

5. The available material of stages following implantation in suckling females shows no evident correlation with either the number of nursing young or the number of embryos being carried. It also is impossible at present to reconcile the development of these embryos with the observed facts regarding the time of parturition in suckling mice.

6. The conflicting evidence from post-implantation stages in suckling females is at present being subjected to further study.

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ON THE POSTNATAL GROWTH OF THE BODY AND OF THE CENTRAL NERVOUS SYSTEM IN ALBINO RATS THAT ARE UNDERSIZED AT BIRTH

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Among the newborn young of various species of mammals there occasionally appear individuals that are undersized and have a very small weight at birth, although they are apparently normal in all other respects. Such individuals are very generally called 'runts' and they are usually discarded by breeders at birth, since it is the popular belief that they never attain normal size and that they are always sterile. Little is known of the true status of these animals, as few attempts have been made to rear them for the purpose of studying their growth processes and reproductive capacity.

In the course of an extensive series of breeding experiments with the albino rat a number of litters have been obtained in which one or more of the rats was very small at birth and weighed much less than the average birth weight for the young of the species, which is about 4.5 grams for the male and 4.3 grams for the female rat (King, '15 a). An examination of these litters some twenty-four hours after birth has shown, as a rule, that the very small individuals were dead, although all of the other members of the litter were alive and vigorous. Thus even under very favorable environmental conditions rats that are much below the average size at birth have little chance, apparently, of surviving even the first few hours of postnatal life; in a state of nature probably very few of them ever live to reach maturity.

In three of the litters examined the undersized young survived the seemingly crucial twenty-four period, and it was found possible to rear them with the other members of the litter and to study their growth in body weight. In order that the small

individuals might have every possible chance for subsequent growth, the young that were not of the same sex as the smallest member were discarded from each litter. This gave two series of female rats, one containing three and the other four individuals; and one series of four males. The rats in the first and those in the third series belonged to the twentieth generation of an inbred strain of albinos in which matings had been made only between brother and sister of the same litter in each generation; the rats in the second series were the offspring of an inbred female (nineteenth generation) and a stock male. For convenience litters with a parentage like that of the second series are designated as 'half-inbred' litters.

The rats in each series were weighed for the first time before they had suckled and then daily for one week. Thereafter the weighings were made at intervals varying from two to fifteen days until the rats were 150 days old when the experiment was ended. In an investigation of this kind it is impossible to obtain the exact body weights owing to the varying amounts of food in the digestive tract at the time that the animals are weighed. To minimize this source of error as much as possible all of the weight records were taken in the morning before the rats had received their daily ration.

Table 1 gives the birth weights of the rats belonging to the three series and also their later body weights at the different ages for which records were taken.

There was considerable variation in the birth weights of the rats in each of the three litters, as is shown in table 1, but in no series was the range of variation as great as that known to occur within the species (King, '15 a). In the first series the heaviest rat (no. 3) weighed but slightly more than the average weight for the female albino rat at birth, yet this weight is 77 per cent greater than the weight of rat no. 1 which had the smallest birth weight that has been found, as yet, in any female rat in our colony. The range of variation in the birth weights of the rats in the second series is much less than that in either of the other series, yet the weight of the heaviest member (no. 4) is 46 per cent greater than that of the smallest member. In the

TABLE 1
Showing the body weights in grams, at different periods from birth to 150 days of age, of three series of albino rats

AGE IN DAYS.....		BODY WEIGHT IN GRAMS																							
		Birth	1d	2d	3d	4d	5d	6d	7d	10d	12d	14d	17d	20d	25d	30d	40d	50d	60d	75d	90d	105d	120d	135d	150d
Series 1 (Females)	Rat No.																								
	1	2.6	3.0	3.4	3.9	4.5	5.1	5.8	6.6	8.9	12.3	14.5	17.2	19.0	28.0	46.4	55.0	76	84	106	113	123	132	133	145
	2	4.4	5.0	5.4	6.0	6.7	7.6	8.4	9.5	12.9	16.0	18.4	21.7	24.5	35.6	54.2	65.0	93	100	120	138	148	150	152	153
Series 2 (Females)	3	4.6	5.2	5.6	6.5	7.4	8.4	9.3	10.6	14.5	17.2	20.0	23.4	26.8	37.3	54.7	62.0	88	97	127	137	149	153	155	158
	1	3.1	3.4	3.8	4.5	5.3	5.9	6.6	7.2	8.8	10.5	12.0	14.7	18.0	19.9	31.6	45.4	55	58	79	110	112	114	124	128
	2	3.9	4.3	4.9	6.0	6.8	7.5	8.1	8.4	10.7	12.2	13.8	16.7	20.5	27.3	34.2	49.4	57	61	95	130	132	139	144	134
Series 3 (Males)	3	4.4	5.0	5.3	6.5	7.4	8.1	8.6	9.3	11.4	12.8	14.5	17.4	21.6	25.9	37.8	52.9	63	69	103	129	136	143	146	149
	4	4.5	5.0	5.4	6.8	7.5	8.6	8.9	9.4	12.2	13.5	14.9	17.7	22.1	29.4	38.7	56.6	71	78	113	137	143	146	154	159
	1	2.7	3.2	3.4	3.7	4.4	4.9	5.7	7.0	10.6	13.3	17.5	21.6	29.0	40.8	50.2	80.9	87	93	142	161	196	203	215	217
	2	3.4	4.0	4.6	5.3	6.0	7.0	8.4	10.4	16.1	19.9	23.7	29.7	34.1	44.9	54.0	83.3	101	111	162	192	223	236	256	263
	3	5.2	5.8	6.5	7.9	8.9	10.6	12.7	14.8	21.8	24.7	28.7	36.6	39.8	51.4	63.5	87.2	115	119	180	209	250	267	282	283
	4	5.3	5.9	6.8	8.0	9.4	11.1	13.4	15.9	22.2	24.8	30.2	36.9	44.1	61.9	73.3	101.3	120	125	182	205	238	250	270	272

third series the birth weights ranged from 2.7 grams to 5.3 grams, the largest individual being nearly twice as heavy as the smallest.

All of these rats followed the normal course of the growth in body weight, as already determined by Donaldson ('06), in spite of the very great differences in their birth weights. Increase in body weight was very rapid during the early days of postnatal life. The rate of growth dropped off somewhat abruptly at about thirty days, and again showed a marked decline at seventy-five days. After the rats reached ninety days of age the period of rapid growth was ended, and the rats gained relatively little in body weight up to 150 days of age when the weighings were discontinued. With few exceptions the body weights of all individuals fall within the range of variation in the body weights of stock albino rats of like age (King, '15 b, table 3). None of the rats that were unusually small at birth, therefore, could properly be considered as 'runts' when they became mature.

The rats that were undersized at birth never succeeded in attaining a body weight equal to that of the other individuals in the same series at any period of their growth, and at successive weighings the actual weight differences between the individuals that were small and those that were heavy at birth tended to increase. This was true for the individuals in each of the three series, irrespective of sex, as is shown by the data in table 1. At the end of 150 days the rats in each series still maintained the same order with respect to body weight that they had at birth, with the exception of rat no. 3 in the third series. This rat overtook its brother, which had a heavier birth weight, when it was ninety days of age and it subsequently kept the lead in body weight until the end of the experiment.

According to the 'standard' tables for the relation of age to body weight and to body length in the albino rat as given by Donaldson ('15), breeding females should have a body length of 193 mm. and a body weight of 186.1 grams when they are 150 days of age; the body length of a male albino rat of the same age should be 207 mm. and the body weight 218.7 grams. Records for the growth in body weight of a selected series of stock albino rats reared in The Wistar Institute animal colony under

environmental conditions similar to those under which the rats used in the present experiment lived (King, '15 b) show that for this group the average body weight of breeding females at 151 days of age is the same as that given by Donaldson, namely 186.1 grams; the average weight of the males of the same age is 244.8 grams, which is 26.1 grams above the computed weight for the male as given in Donaldson's tables.

The average body weight of all of the females used in this experiment was 146.7 grams, and that of the males was 258.7 grams, when the animals were 150 days old. The females, as a group, are too light in weight for their age, while the males are much too heavy, whichever of the above series of records is taken as a standard for comparison.

The fact that the females were either strictly inbred or half-inbred does not account for their small size, since in the strain of albinos from which these rats were taken inbreeding has increased rather than diminished the average body weight of both males and females (Popenoe, '16). Investigations made by Watson ('05) have shown that female rats that are allowed to breed are heavier at a given age than non-breeding females. It is probable that the low weight of these females is due, in some measure at least, to the fact that the rats were never mated (the stock females whose weights were given for comparison were all breeding animals). The relatively large size of the males in the third series can be attributed to the fact that the animals were from a selected inbred strain.

The average daily percentage gain of these rats in body weight during the period covered by the experiment is shown in table 2.

The percentage values for weight increase, unlike the weight data, show no definite order with respect to the birth weights of the individuals concerned. At some periods the rat which had the smallest birth weight shows a greater daily percentage gain in weight than any other member of the same series; at other periods the weight excess is in favor of the individual with the heavier birth weight (table 2).

If, instead of considering all of the percentage values in a given series, only the data for the two individuals that had the extreme

TABLE 2
Showing the average daily percentage weight increase of three series of albino rats (weight data in table 1)

		DAILY PERCENTAGE INCREASE IN BODY WEIGHT																								150 days over birth
	RAT NO.	1st day	2nd day	3rd day	4th day	5th day	6th day	7th day	7-10 days	10-12 days	12-14 days	14-17 days	17-20 days	20-25 days	25-30 days	30-40 days	40-50 days	50-60 days	60-75 days	75-90 days	90-105 days	105-120 days	120-135 days	135-150 days		
Series 1 (Females)	1	15.38	13.33	14.70	15.38	13.33	13.72	13.79	11.61	19.32	8.94	6.20	3.45	9.47	13.14	1.85	3.81	1.05	1.74	0.44	0.59	0.48	0.05	0.60	5476.92	
	2	13.63	8.00	11.11	14.66	13.43	10.52	13.09	11.93	12.01	7.50	5.91	4.30	9.06	10.44	1.99	4.30	0.75	1.33	1.00	0.48	0.09	0.09	0.04	3377.27	
	3	13.04	7.69	16.07	13.84	13.53	10.71	13.97	12.68	9.31	8.13	5.66	4.84	7.83	9.32	1.33	4.19	1.02	2.04	0.52	0.58	0.18	0.09	0.04	3313.04	
	4	11.11	8.00	25.92	10.39	14.66	3.47	5.61	9.92	5.32	5.18	6.26	8.28	6.60	6.39	4.62	2.54	0.98	2.99	1.41	0.29	0.14	0.46	0.17	3433.33	
Series 2 (Females)	1	9.67	11.76	18.42	17.77	11.32	11.86	9.09	7.40	8.65	7.14	7.50	7.48	2.11	11.76	4.39	2.11	0.54	2.42	2.61	0.12	0.12	0.58	0.21	4029.03	
	2	10.25	13.95	22.24	13.33	10.39	8.00	3.70	9.12	7.00	6.55	7.00	7.58	6.63	5.05	4.44	1.53	0.70	3.71	2.45	0.10	0.38	0.24	0.46	3335.89	
	3	13.63	6.00	22.64	13.84	9.46	6.17	6.97	7.52	6.14	6.64	6.66	8.04	3.98	9.19	3.99	1.90	0.53	2.81	6.80	0.36	0.34	0.14	0.14	3286.36	
	4	11.11	8.00	25.92	10.39	14.66	3.47	5.61	9.92	5.32	5.18	6.26	8.28	6.60	6.39	4.62	2.54	0.98	2.99	1.41	0.29	0.14	0.46	0.17	3433.33	
Series 3 (Males)	1	18.51	6.25	8.82	18.91	11.36	16.32	22.80	17.14	12.73	15.79	7.80	11.41	8.14	4.60	6.11	0.75	0.69	3.51	0.89	1.45	0.24	0.39	0.06	7937.03	
	2	17.64	15.00	15.21	13.40	16.66	20.00	23.80	18.27	11.86	9.54	8.43	4.93	6.33	4.05	5.40	2.12	0.99	3.06	1.23	1.07	0.39	0.56	0.19	7635.29	
	3	11.53	12.07	21.53	12.65	19.10	19.81	16.53	16.43	6.65	8.09	9.17	2.91	4.51	4.70	3.73	1.90	3.53	4.11	0.71	1.30	0.45	0.31	0.02	5323.07	
	4	11.32	15.25	17.64	17.50	18.08	20.72	18.65	13.20	5.85	10.88	7.39	6.50	8.13	3.68	3.82	1.84	0.42	3.04	0.84	1.04	0.34	0.53	0.05	5032.07	

birth weights are compared, the results obtained seem consistent enough to be significant. In the total of 69 records for the three series, 42, or nearly two-thirds, show that the daily percentage weight increase is greater for the individuals with a low birth weight than for those with a heavy birth weight. In other words, regardless of sex, rats that are very small at birth tend to grow more rapidly than do rats that have a heavy birth weight, although their actual body weights are always less at any given period.

On computing the percentage increase in body weight at 150 days over the birth weight for the various individuals it was found that the rats that were small at birth had gained a greater amount than had the rats that were heavy at birth. For each series, as shown in the last column in table 2, these percentage values stand in inverse order to that of the birth weights, with the one exception in the second series (rat no. 4).

The results of this investigation are in accord with those obtained by Dunn ('08) in her study of the weight increase in a 'group' of seven albino rats (three males and four females) which had very unlike body weights when they were fourteen days old. Dunn found that, with one exception, the order relation of the weight at fourteen days of age was maintained until the end of the experiment; the rats having the heaviest initial weights were also the heaviest at sixty-six days of age when the weighings were discontinued. The lighter rats, on the other hand, while putting on less absolute weight, had gained at the end a greater percentage of their original weight than had the individuals with the heavier initial weight.

In addition to the undersized young which, as shown above, are capable of developing into adults which are only slightly below normal, a litter sometimes contains individuals of a much lower grade to which the term 'runt' properly applies. In these individuals, which apparently are indistinguishable from the other young at birth, the normal action of the growth factors is inhibited from the very beginning of postnatal life by unknown constitutional causes, not by environmental conditions. When the young rats are old enough to leave the nest the runts can

easily be distinguished from the other members of the litter, not only because of their very small size, but also because of their slower movements and apparent lack of normal vitality. Runts grow slowly for a certain time, but no matter how favorable the external conditions, they never exhibit normal vigor and they are always dwarfed and stunted in their body growth. In such animals growth is not merely retarded, as it is in the case of rats experimentally stunted (Hatai, '07; Osborne and Mendel, '14), but it is permanently checked at an early age. Several attempts have been made in our colony to increase the size of runts by special feeding, and to breed them for the production of a dwarfed race of rats. Only a few litters could be obtained from such stock, and these contained a very small number of young which were puny from birth and which died at an early age. The reproductive powers of these animals are apparently never developed in a normal way, as the males rarely mate and most of the females are sterile.

All of the rats used in this study were killed at the end of 150 days, the body weights and body lengths determined, and the brains and spinal cords removed and weighed. This was done in order to ascertain whether the central nervous system in adult rats that were small at birth bears the same relation to body weight and to body length as that found in adult individuals that were of average size, or above, at birth.

Table 3 gives the body lengths of the individuals, the observed weights of the brains and of the spinal cords, and the brain and cord weights corrected according to table 68 in 'The rat: data and reference tables' (Donaldson, '15) which gives the computations for the 'standard' weights of the central nervous system in albino rats of various body lengths. In addition to the above data, table 3 shows the percentage deviations of the observed weights of the brains and of the spinal cords from the corresponding standard weights.

It is evident, from the percentage values given in the fifth and in the eighth columns of table 3, that the observed weights of the brain and of the spinal cord in all of the rats are considerably below the standard weights for these organs in animals

TABLE 3

Showing the body lengths, with the observed and the 'standard' weights for the brain and for the spinal cord, of the albino rats whose weight data are given in table 1

	RAT NO.	BODY LENGTH IN MM. (150 days)	OBSERVED BRAIN WEIGHT	STANDARD BRAIN WEIGHT FOR BODY LENGTH	PER CENT DEVIATION OBS. BRAIN WEIGHT FROM STANDARD	OBSERVED CORD WEIGHT	STANDARD CORD WEIGHT FOR BODY LENGTH	PER CENT DEVIATION OBS. CORD WEIGHT FROM STANDARD
Series 1 (Females)	1	183	1.461	1.773	-17.5	0.455	0.524	-13.1
	2	185	1.611	1.782	-9.5	0.473	0.532	-11.0
	3	182	1.679	1.768	-5.0	0.499	0.520	-4.0
Series 2 (Females)	1	175	1.511	1.735	-12.9	0.418	0.492	-15.0
	2	184	1.616	1.778	-9.1	0.467	0.528	-11.5
	3	192	1.653	1.814	-8.8	0.485	0.560	-13.4
	4	188	1.643	1.796	-8.5	0.475	0.544	-12.6
Series 3 (Males)	1	198	1.697	1.849	-8.1	0.529	0.556	-4.8
	2	210	1.765	1.903	-7.2	0.579	0.603	-3.9
	3	217	1.955	1.933	+1.1	0.631	0.630	+0.2
	4	212	1.999	1.911	+4.6	0.669	0.611	+9.4

of like body length, except in the case of the two largest males where the observed weights slightly exceed the standard weights. That the weights of the central nervous system in all individuals of a given litter, having the same sex and about the same body weight, should deviate from the standard weights in the same direction was not an unexpected result. In the rat variation within the litter unit is usually in the same direction and much less than that in the general population as regards body weight (Jackson, '13; King, '15 b), and doubtless this rule holds for the central nervous system and other organs as well. In every series, as shown in table 3, the rats with the smallest birth weights are the ones whose brain and cord weights show the most marked deviations from the standard weights, regardless of their body length and body weight with which the weight of the central nervous system is, as a rule, closely correlated. Thus, in the first series, female no. 1 had a body length of 183 mm. and a body weight of 145 grams while female no. 3 was shorter and heavier,

yet in the former individual the brain was 17.5 per cent and the cord 13.1 per cent below the corresponding standard weights; in the latter individual the brain and cord weights were only about 5 per cent less than the standard weights. All of the rats in the second series had cord weights that showed relatively greater deviations from the standards than did the brain weights; the lowest weight for both brain and cord being found in the rat that had the shortest body length and the smallest body weight (no. 1). The most interesting result is found on comparing the records for the rats belonging to the third series. The brains of the two rats that had birth weights much lower than the average birth weight (nos. 1 and 2) were each about 8 per cent less than the standard and the cord weights showed a minus deviation of some 4 per cent from the standard, yet in body measurements rat no. 1 was below and rat no. 2. was above the average for stock males of like age. The brain and cord weights of their brothers, each of which was unusually heavy at birth, were above the computed standard weights for the central nervous system in animals of like body length.

That the postnatal growth of the central nervous system is influenced to some extent by the factors that determine the size of a rat at birth seems to be a conclusion warranted by the analysis of data given above.

SUMMARY AND CONCLUSIONS

The results obtained in this investigation seem to indicate that the undersized individuals which are sometimes found in a newborn litter of rats are not necessarily 'runts' in the generally accepted use of that term. Some of these small individuals, as shown above, attain an adult size that not only is within the normal limits of variation in the body weights of standard stock rats of like age, but may even exceed the average body weight of a large number of stock animals (rat no. 2, series 3).

All rats in a litter are not born with a like capacity for growth, as the data in table 1 indicate, and even when environmental conditions are as favorable and as uniform as it is possible to make them, individuals having unlike birth weights show marked differences in their rates of growth from birth to the adult state.

In the cases studied the individuals having a small birth weight seemed to possess a very great capacity for growth from the very beginning of postnatal life. Female no. 1 of the first series increased 2 per cent more in body weight during the first twenty-four hours after birth than did either of her sisters; for the same period the gain in body weight of the two smallest males in the third series was over 6 per cent more than that of their brothers. In the second series, however, the weight increase for the first day was greater for the rats that were heavy than for those that were small at birth.

With an early acceleration in the rate of growth there is seemingly correlated an early cessation in the extent of growth, as in the adult state individuals that were undersized at birth are always smaller than the other members of the same litter. On the other hand, rats with a heavy birth weight tend to grow more slowly at first than do the smaller individuals, but they continue to grow for a longer time and eventually reach a greater size. This rule seems to apply to females as well as to males.

Not only does body weight at birth indicate the probable capacity of the individual for subsequent growth, but it also indicates the probable size of the central nervous system, since rats that are undersized at birth tend to have a much smaller central nervous system when they become mature than do other rats. The factors, whatever their nature, that determine the body size of a rat at birth seem to have a marked effect on the subsequent postnatal development of the individual, influencing the ultimate body weight as well as the size of the central nervous system.

A very small weight at birth indicates that a rat has a handicap in its organization that environment, however favorable, cannot overcome. Such animals, although they appear vigorous and healthy during their growth period and after reaching the adult state, are unquestionably sub-normal in regard to the size of the body and of the central nervous system. If allowed to breed these rats would probably produce young having a weaker constitution than their own, and from such stock one would ultimately get 'runts' and an increasing tendency towards sterility that would soon bring disaster to the colony.

Judging from the results obtained in this study a newborn litter of rats may contain individuals of three kinds as regards their inherent capacity for body growth. As a rule, only young rats having a normal birth weight and a normal capacity for growth are found in a small or medium sized litter produced by a female rat in good physical condition. Occasionally rats are born which have a very small birth weight, and in these individuals, if they are able to survive, the growth capacity is lessened to some extent but not sufficiently to prevent them from being classed as 'normal' after they have reached maturity. If a litter is very large, or if the mother is not in good physical condition during the gestation period, some of her young may be born with their growth capacity so impaired that it is impossible for them to grow beyond a certain stage. These individuals are true 'runts' and, fortunately, they are lacking in reproductive vigor as well as in growth capacity so that they are usually unable to reproduce their kind and so prove a menace to the colony in which they live.

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MOUNTING SPECIMENS UNDER PETRI DISHES AND CLOCK GLASSES

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Since obtaining a suitable cement for the sealing of square museum jars, the mounting of thin sections of pathological and anatomical specimens under Petri dishes and clock glasses has been made both easy and cheap. Several years ago these mounts attracted a great deal of attention, being put on the market as paper weights, etc., but were gradually adopted for the exhibition and display of museum specimens. The method, however, being patented was expensive and beyond the reach of the average museum; besides it was necessary to send your material for mounting to the manufacturer. In 1906 Dr. Hutchinson of the Royal Victoria Hospital, Montreal, read a paper before the British Medical Association at Toronto on this method; but unfortunately he did not have a suitable cement and the process was slow and laborious.

METHOD

The fluid used for mounting should be brought to a boil in the same dish that is to be used for mounting and allowed to stand over night, or until cool, to get rid of as much air as possible. The dish should be deep enough to come well up over the mount to allow of easy manipulation of both Petri dish and specimen. Get a Petri dish of suitable size to hold the specimen so that when the sheet of glass which is to form the cover of the mount is in position it will not quite touch it. Great care must be taken that the Petri dish or clock glass fits perfectly on the base and does not rock.

In placing the Petri dish in the mounting fluid, do so without causing any air bubbles. When the dish and fluid are ready, wash the specimen in several changes of the same fluid that you are mounting in, to get rid of any loose particles or dirt. In the last change of fluid, work out all air and remove quickly to the Petri dish, face downwards, again eliminating air bubbles. A small piece of looking-glass in the bottom of the mounting dish is very convenient, as by tipping the Petri dish over and on its side slightly, any bubbles under the specimen may be seen.

The specimen now being in position, put on the base or cover and remove from the mounting-dish, holding it firmly so that it cannot slip

and admit air while turning the mount right side up. Over the junction of Petri dish and cover pour hot cement¹ to the thickness of about one quarter of an inch, and allow the mount to stand on a flat surface for a few days, or until the cement is perfectly adherent to both Petri dish and cover. If after several days it is desired to finish the mount, remove any bubbles of fluid between the cover and the cement with a very hot knife, working the knife to the outer edge of the base. This must be carefully done, and the knife kept hot enough so that the cement will be kept liquid and not be drawn away from the Petri dish. All the fluid must be removed from under the edge of the Petri dish in this way.

The best results are obtained, however, by allowing the mount to stand for several weeks when most of the excess fluid will work out by itself. I usually mount several dozen specimens at a time and let them stand until they are ready to finish. If any air bubbles should happen to get under the edge of the Petri dish they will have to be worked into the mount in the same way that fluid is removed, only work your knife inwards instead of outwards.

Even with the greatest care air is often retained in the specimen itself, and only detected after the mount has stood for several days. Should the bubbles be very small they will quite frequently be absorbed by themselves; if not, shake the mount until they are all in one large bubble, and resubmerge in mounting fluid which must be heated as hot as you can comfortably stand your hand in. When the cement has become pliable enough so that it is possible to move the Petri dish with the fingers, insert the point of a sharp knife between the dish and base, gently forcing them apart and allowing a little fluid to enter. The mount must then be tilted on its side to bring the air bubble to the opening, where it will escape when you remove the point of the knife; then press the Petri dish up against the base, expelling all superfluous fluid. Close the hole by pressing the soft cement together with the fingers.

Air bubbles may have to be removed several times depending on the specimen. Sections of lung give the most trouble. When sure that there are no more bubbles in the mount and that all fluid has been removed from between the cement base and the Petri dish, coat the cement over to the thickness of about one-eighth of an inch with refined asphalt, being careful that it does not burn; I usually melt it in a tea-spoon over a Bunsen burner. When the asphalt has been all applied, reheat the whole with a very hot knife and apply a bezel ring which must be heated red hot, and pressed down into the cement so that the lower edge will rest upon the base. Clean with gasolin and polish with bon ami soap.

The Petri dishes used are manufactured specially from 2 to 3 mm. thick, as the ordinary ones sometimes break with the expansion and

¹ See Muir and Judah, Sealing of museum jars. Bulletin 5, Inter. Assoc. Med. Mus., page 87.

contraction of the fluid. The bezel rings which are used to give a finished appearance to the cement can be made out of any metal that will stand being heated red hot. They are not, however, absolutely necessary, as you can use a sheet of cardboard with a hole cut in the centre and passe partout the edges. Dr. Higgins of the Experimental Farm, Ottawa, has a very convenient cardboard case into which he slips the mount. If a bezel ring is to be used, the base or cover should be made out of plate glass with the edges bevelled.

Clock glasses are inferior to Petri dishes for this method because they magnify and distort the specimen.

The following list of sizes have been found to answer all requirements. While 36 in number they only require ten different sized bezel rings and plate glass bases, a desired advantage when the glass ware is made to order.

Sizes (Outside measurements)

SIZE NO.	WIDTH	HEIGHT	SIZE NO.	WIDTH	HEIGHT
	<i>cm.</i>	<i>cm.</i>		<i>cm.</i>	<i>cm.</i>
1	18	3	19	8	2.5
2	18	2	20	8	2
3	18	7	21	8	1.5
4	16	3	22	8	1
5	16	2	23	6	2.5
6	16	1.5	24	6	2
7	14	3	25	6	1.5
8	14	2.5	26	6	1
9	14	2	27	4	2.5
10	14	1.5	28	4	2
11	12	3	29	4	1.5
12	12	2.5	30	4	1
13	12	2	31	3	2
14	12	1	32	3	1.5
15	10	2.5	33	3	1
16	10	2	34	2	2
17	10	1.5	35	2	1.5
18	10	1	36	2	1

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